"A STUDY ON ROLE OF HBsAg QUANTIFICATION IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION AND PREDICTING ITS RESPONSE TO TREATMENT"

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CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON ROLE OF HBsAg QUANTIFICATION IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION AND PREDICTING ITS RESPONSE TO TREATMENT"** submitted by **Dr. Balaji G** to the Faculty of Medical Gastroenterology, the Tamilnadu Dr.MGR Medical University, Guindy, Chennai-600032, in partial fulfillment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by him under my direct supervision and guidance, during the academic year 2012 to 2015.

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DECLARATION

I **Dr. Balaji G** declare that I carried out this work on **"A STUDY ON ROLE OF HBsAg QUANTIFICATION IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION AND PREDICTING ITS RESPONSE TO TREATMENT"**at the Department of Medical Gastroenterology, Govt.Peripheral Hospital and Kilpauk Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any university, board either in India or abroad.

This is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the D.M. Degree examination in Medical Gastroenterology.

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ABBREVIATIONS

AALSD : American Association for Study of Liver disease

AFP : alpha feto protein

ALT : Alanine Transaminase

Anti HBc : Antibody to Hepatitis C Core antigen

Anti Hbs : antibody to Hepatitis B surface antigen

AST : Aspartate Transaminase

cccDNA : Covalently closed circular DNA

CHB : Chronic Hepatitis B

DNA : Deoxyribo Nucleic Acid

HBeAg : Hepatitis B e antigen

HbsAg : Hepatitis B surface antigen

HBV : Hepatitis B Virus

HCC : Hepatocellular Carcinoma

HCV : Hepatitis C Virus

HIV : Human immunodeficiency virus

INR : International Normalized Ratio

MELD : Model for End Stage disease

NS : Not significant

NUC's : Nucleotide analogues

ORF : Open Reading Frames

Peg-IFN : pegylated Interferon

qHBsAg : quantification of Hepatitis B surface antigen

RNA : Ribo Nucleic Acid

ROC : Receiver Operating characteristics

RT : Reverse transcriptase

SVR : Sustained Virological response

ULN : Upper limit of normal

USG : Ultrasonography

ABSTRACT

Aim: To study the correlation of HBsAg quantification levels with HBV DNA in patients with chronic hepatitis B infection.Also to study the role of qHbsAg levels in predicting the response to antiviral therapy in chronic hepatitis B infection.

Materials and methods : one year prospective study conducted between January 2014-2015 at department of digestive health and diseases, kilpauk medical college,chennai. All patients of chronic hepatitis B infection were included in the study. Baseline liverfunction tests, HbsAg quantification and HBVDNA levels were done. In treatment group the above values are repeated every 12 weeks for 36 weeks.

Results : Total of 160 patients were included and among them 24 were in treatment group. The results are as follows. In the treatment naïve group, serum HBsAg levels are higher in HBeAg positive group than HBeAg negative group with a mean of 4.25logIU/ml and 2.81logIU/ml respectively . so qHBsAg levels had significant correlation with HBVDNA levels in HBeAg positive group (P <0.001) but not in HBeAg negative group. Serum qHBsAg levels also differentiates immune-tolerant from immune-clearance phase with mean values of 4.59logIU/ml and 3.74logIU/ml respectively(P=0.038).We observed that Serum

HbsAg levels are higher in active chronic hepatitis B group than inactive carriers with a values of 4.20logIU/ml and 2.64logIU/ml respectively (P=0.002). From the above ROC curve, serum HBsAg level of 3.01logIU/ml indicates high chances of replicative state with 96% sensitivity and 76% specificty. In treatment with NUC's we observed decline in serum qHbsAg levels are slow and less pronounced that HBVDNA in both HBeAg positive &HBeAg negative group.

conclusion: High serum qHBsAg levels has a good correlation with HBV DNA levels in HBeAg positive than HBeAg negative patients. Single point estimation of qHBsAg levels can predict replicative state and can act as a surrogate marker for the replicative state. Higher qHBsAg levels also differentiates inactive CHB from active CHB and can replace HBV DNA levels in differentiatin the two. Estimation of qHBsAg is easy and cost effective. Serum qHBsAg levels decline slowly with NUC's than DNA levels and decrease in serum qHBsAg levels does not correlate with decrease in HBV DNA levels.

INTRODUCTION

Hepatitis B infection is a major health problem affecting approximately about 350 million globally. Annually about 600,000 people die from hepatitis B-associated liver disease [1] . Approximately 0.8–1.4 million individuals are chronically infected with HBV in the United states alone [2]. Since the discovery of surface antigen , HBsAg remained as the gold standard for the diagnosis of HBV infection[3]. Though the vital role of HBsAg quantification was discovered long ago, its usefulness was not studied or observed. The reason may be due to lack of simple test and it's availability unlike recent days. Hence estimation of qHBsAg levels use was restricted in clinical practice . The knowledge behind the usefulness of HBsAg quantification based on that there is strong and positive association with covalently closed circular (ccc) DNA which is the template for viral replication in the hepatocytes [4].

Serum HBsAg levels comprises of virions and non-infectious HBsAg particles(filaments &spherical) . During the course of the disease, Filaments decrease and goes in parallel with virions where as the spherical particles remain stable and in moderate excess in low viraemic HBeAg negative carriers. Thus, HBsAg production varies both quantitatively and qualitatively over time and during different phases of the infection[5]. Serum HBsAg levels starts declining gradually from the immune-tolerant (IT) to immune clearance(IC) phase with higher values in immunotolerant phase and lower levels in immune clearance phase. There is no clear cut-off values in each phases as various studies showed different values. In immune-tolerant patients, the median values showed variability i.e 5 logIU/ml from European study , 4.9 logIU/ml from Hong Kong study and only 4.5 logIU/ml from Asia study [7].

Also there is wide fluctuations in DNA levels and ALT levels in IT & IC phase which makes it difficult to differentiates IT from IC phase ,but serum HBsAg levels can differentiate IT from IC phase by higher qHBsAg levels in IT phase than IC phase , the mean serum HBsAg level was 4.96 log IU/ml vs. 4.37 log IU/ml, in IT and IC phase respectively [6]. And the rate of decline in qHBsAg levels varies at each phases of HBV life cycle. Studies have shown that decline in HBsAg

is less pronounced in HBeAg-negative patients than in HBeAg-positive patients . HBsAg quantification levels also helps in monitoring the treatment response and guides in stopping the therapy. Studies have shown that qHBsAg levels of more 1 logIU/ml decline at 12 weeks from base line had sensitivity of 78% , specificity of 96% in predicting the sustained response. so HBsAg levels can guide in stopping of antiviral therapy in CHB[8]. So , this study is done to evaluate the role of HBsAg quantification in treatment naïve chronic hepatitis B infection and also in predicting the treatment response.

OBJECTIVES OF THE STUDY

1. Correlation of HBsAg quantification levels with HBV DNA levels in patients with chronic hepatitis B infection.

2. Role of hepatitis B surface antigen quantification in predicting the response to antiviral therapy in chronic hepatitis B infection.

REVIEW OF LITERATURE

Hepatitis B surface antigen (HBsAg) was discovered by Blumberg in 1965, and since then HBsAg has been used as the goldstandard for the diagnosis of hepatitis B infection[1]. Worldwide over 2 billion people are infected with HBV, among those 400 million are chronically infected. Chronic hepatitis B infection can progress to complications like cirrhosis, decompensation of liver and hepatocellular carcinoma. Although most of them will not develop these complications, only 15- 40% will develop complications over a period of many years[9].

HEPATITIS B VIRUS:

The hepatitis B virus is a DNA virus belongs to the hepadnaviridae family. Hepatits B virion is also known as the Dane particle which is 42-nm. It contains small DNA genome inside nucleocapsid which is surrounded by an outer envelope called surface antigen(HBsAg) [10].

Epidemiology and Prevalence :

The prevalence of Hepatitis B infection varies widely and globally due to differences in the predominant mode of transmission and the age at infection. The regions are divided based on rate of prevalences. In highly prevalence regions like Southeast Asia (excluding Japan) and sub Saharan Africa, the commonest mode of infection is by perinatal transmission accounting for 40-60 % of chronic B infection(CHB). In the intermediate prevalent countries India, Japan, the Middle East and some parts of southern and eastern Europe, CHB accounts for 20% with a carrier rate of 3-5%. Horizontal mode of transmission is the most common mode in these regions. Countries like Australia, North America, western Europe, and some regions of South America has a low prevalence with carrier rate of 0.1- 2%. The major modes of transmission in these regions are sexual transmission and injection drug users .[11-13]

The significant impact on the clinical outcome depends on the age at infection occurs. The rate of chronic infection is 90% in infants infected at birth, 25–50% if infected between 1 and 5 years, and < 5% if infected during adult life [14-17].

Molecular biology of Hepatitis B virus:

❖ **Molecular biology:**

The genome of Hepatitis B virus has four important open reading frames (ORFs). HBV genome has a compact design with a several genes on it. These genes overlap with each other and they encode different viral proteins by using the same DNA molecule . The genes which encode the open reading frames are the core, surface, X, and polymerase genes.

The largest gene among these four is Polymerase gene with approximately 800 amino acids and this gene overlaps with the entire length of the surface ORF. This polymerase gene Codes for DNA polymerase/reverse transcriptase and the function of this gene includes packing and DNA replication((includes RNA- and DNA-dependent DNA polymerase, RNase H activities and priming) . The surface gene 'S' codes for the small surface protein called HBsAg. The preceding pre-S1 and pre-S2 proteins in ORF-S along with S protein codes for small[S], medium [M] and large[L] proteins. These proteins functions as viral recognition by hepatocyte receptors.

Among these small, medium and large proteins are expressed on the surface of Dane particle. Where as the small surface protein is expressed in subviral particles. The core gene 'C' codes for the hepattis B core

nucleo-capsid protein (HBcAg), which is important in viral packaging and production of HBeAg. The X gene encodes the HBx protein, which has a potent transcriptional and transactivating properties and may be important in hepatic carcinogenesis.

The hepatitis B virus replication occurs through an RNA intermediate with the help of active viral reverse transcriptase/polymerase enzyme. Hepatitis B virus has higher mutation rate than any other DNA viruses (an estimated 10^{10} to 10^{11} point mutations per day).[18] HBV genomic sequencing has identified a large number of mutations within the genome, of which many are silent or does not alter the amino acid sequence of encoded proteins. In view of of genomic overlap, some of the silent mutations in one ORF may result in an amino acid substitution in an another overlapping ORF (for example , from polymerase gene to surface gene),which may be the cause for variability in response to treatment, but clinical implications are uncertain as the data is lacking on this[19].

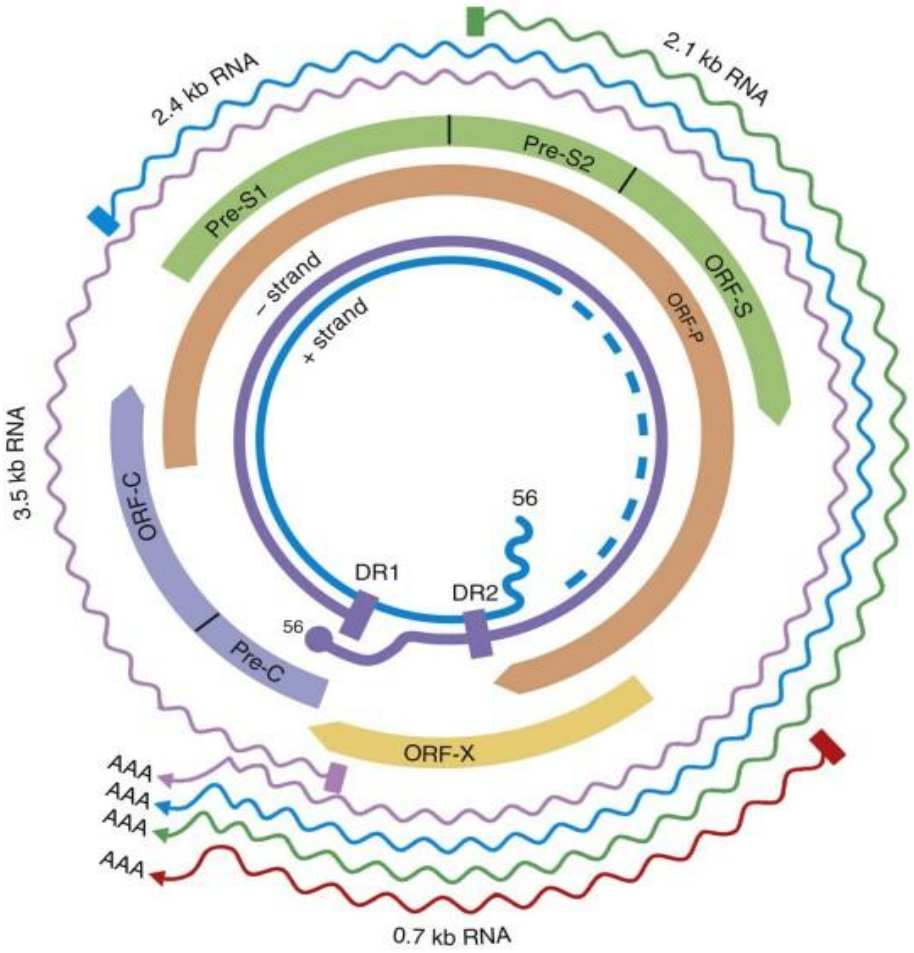


figure 1:open reading frames(ORF's) of genome with major transcripts (wavy lines)

❖ THE LIFE CYCLE OF HBV:

The life cycle begins with the attachment of the virus to the hepatocyte but the receptor for the virus attachment to the hepatocyte has not been identified. The initial phase is attachment of virion to host cell and enters inside by fusion of viral & host membranes. After entering the virus into the hepatocyte, it undergoes uncoating, and then Hepatitis B virus genome enters the nucleus. Following which repairing of the single-stranded DNA strand and formation of the covalently closed circular (ccc) DNA template occurs. Then the Viral transcripts are formed for the hepatitis B surface antigen (HBsAg), DNA polymerase, X protein, and RNA pregenome. The RNA pregenome and DNA polymerase are incorporated into the maturing nucleocapsid and removed after translation.

The surface protein enveloping process occurs in the endoplasmic reticulum. Some of the nonenveloped nucleocapsid re-circulates back to the nucleus, and the cycle begins again. Excess tubular and spherical forms of HBsAg are secreted in great abundance.

S protein is synthesized in the endoplasmic reticulum, where monomer aggregates that exclude host membrane proteins subsequently bud into the lumen as subviral particles. When formed, HBsAg undergoes

glycosylation in the endoplasmic reticulum and the Golgi apparatus. Noninfectious subviral particles (spherical and filamentous forms of HBsAg) are secreted in great abundance when compared with mature virions [19]

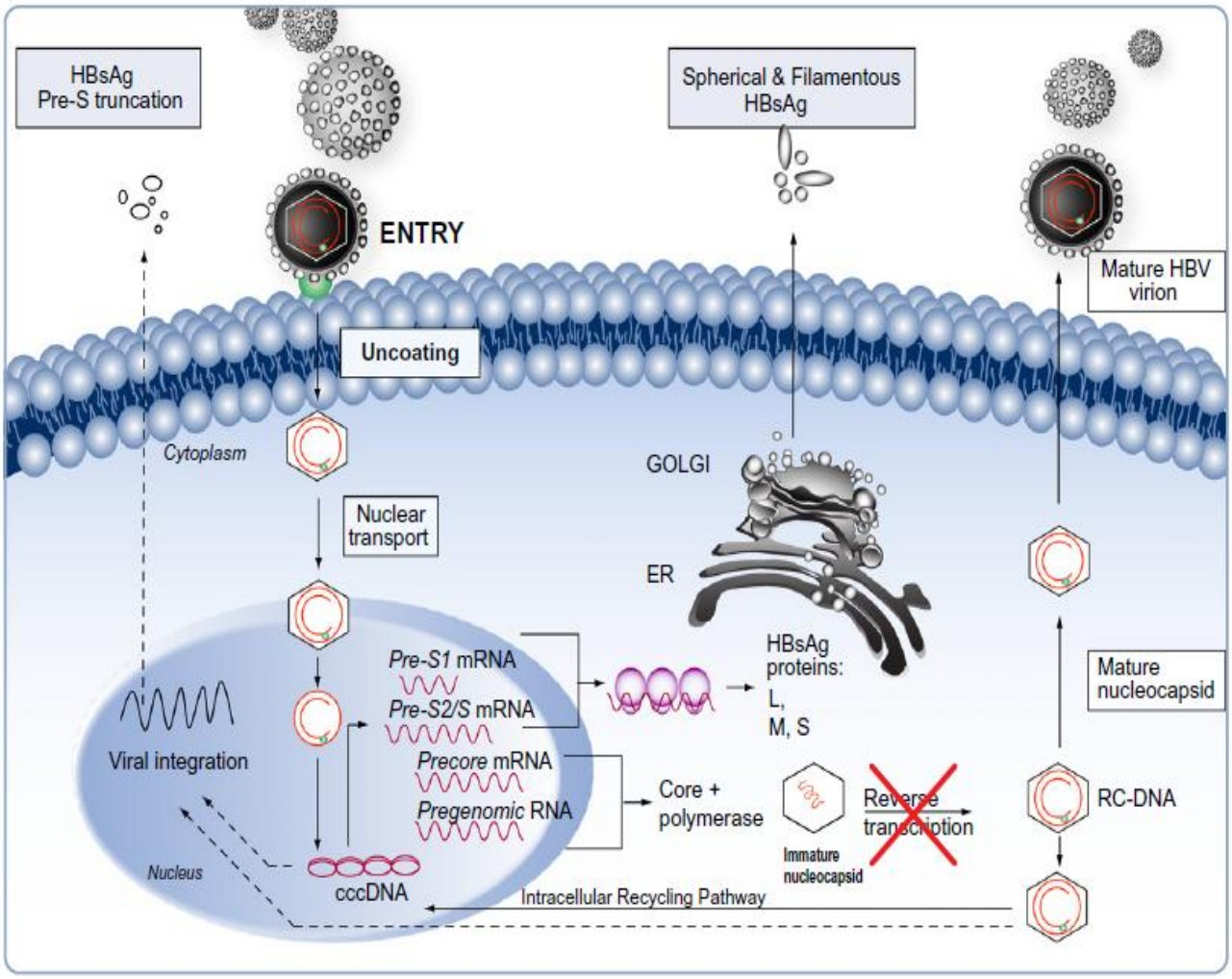


Figure 2: life cycle of Hepatitis B virus

- Hepatitis B Virus Genotypes[20]:

There are 8 genotypes identified so far from A to H based on their nucleotide sequence, distributed all over the world with wide variation. The significance in identifying these genotypes are due to varied response to therapy(mainly interferon) and plays a vital role in determining the disease activity and risk of progression of the disease. In India the predominant genotype is 'D', where as the other part of Asian countries genotype 'B' and genotype 'C' are predominant.

- HBV serotypes :

HBV serotypes are classified based on antigenic determinant on the small S protein. The common determinant is 'a' and additionally there are two pairs of mutually exclusive allelic antigens i.e 'y' versus 'd' and 'w' versus 'r'. Thus, there are 4 possible major serotypes includes, ayw, ayr, adw, adr.

The details of the genotypic distribution and their clinical association with the disease process are described in the following table .

Table 1:

Geographic Distributions
A: Northwestern Europe, North America, Central Africa
B: Southeast Asia, including China, Japan, and Taiwan (prevalence is increasing in North America)
C: Southeast Asia (prevalence is increasing in North America)
D: Southern Europe, Middle East, India
E: West Africa
F: Central and South America, United States (Native Americans), Polynesia
G: United States of America, France
H: Central and South America

Continuation of table 1:

Proposed Clinical Associations
Time to HBeAg seroconversion and probability of HBsAg loss: B < C Response to interferon-α: A > B ≥ C > D Precore/core promoter mutant frequency: precore mutation not selected with A and F Liver disease activity and risk of progression: B < C Evolution to chronic liver disease: A < D Hepatocellular carcinoma risk: B > C in younger age group in Taiwan but B < C in older age group in Japan

HEPATITIS B SURFACE ANTIGEN KINETICS :

HBsAg is the glycosylated envelope protein of the mature HBV virion. Three HBsAg proteins exists namely small (S), medium (M),and large (L) proteins. Apart from virions ,serum HBsAg levels derives mainly from non-infectious HBsAg particles (20 nm diameter filaments) which does not contain viral nucleic acids and exceed virions by a factor ranging from 10^2-10^5. [21] HBsAg derives mainly from the intrahepatic viral minichromosome (cccDNA) by translation of specific RNAs that are distinct from pregenomic RNAHBV replication occurs via the pregenomic RNA (pgRNA),a separate RNA transcript, therefore the HBsAg secretory follows a distinct pathway from viral replication pathway distinct processes within the hepatocyte[22].

The ratios between defective HBsAg particles and virions are not stable, but change over time. Filaments and spherical particles are produced in large excess in highly viraemic hepatitis B ‘e’ antigen (HBeAg)-positive carriers. While filaments decline in parallel with virions, spherical particles remain in moderate excess in low viraemic HBeAg negative carriers.

Thus HBsAg production varies both quantitatively and qualitatively over time and appears to be dynamically regulated during different phases of the infection[23,24].

From the diagnostic perspective, it is important to appreciate that HBsAg quantification detects all three forms of circulating HBsAg.

The antibodies used in the quantitative enzyme immunoassays target epitopes in the S protein. Therefore not capable of distinguishing between virion-associated HBsAg, subviral particles and HBsAg produced from integrated sequence[24]. Currently, there are two commercialized assay that can measure the HBsAg quantification, the Architect QT assay and the Elecsys HBsAg II Quant assay [25].

- **Natural history of surface antigen(HBsAg):**

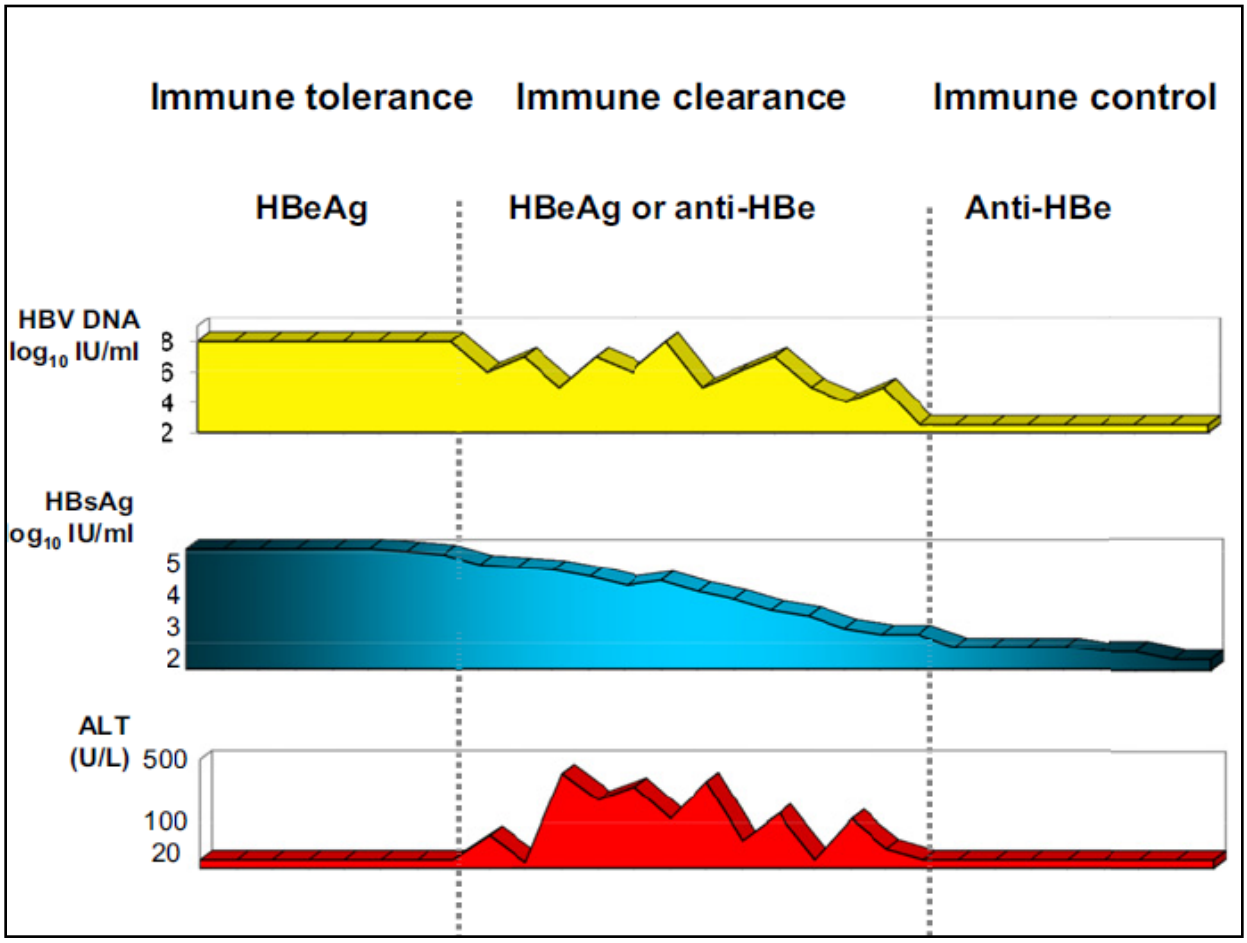
Studies have shown that HBsAg production varies both quantitatively and qualitatively over time and during different phases. Their decline in their levels also varies progressively from the immune-tolerant to immune clearance phase. Various studies have reported that ,higher and more stable HBsAg levels in HBeAg-positive immune-tolerant carriers, with values

about half a log higher than immune clearance phase . HBsAg titers can correlate with serum HBV DNA and intrahepatic cccDNA levels, but vary in different phases of disease[26] .

There is a strong correlation between HBsAg , cccDNA and serum DNA levels in HBeAg-positive patients, where as in HBeAg-negative patients DNA & cccDNA levels are reduced relatively but HBsAg titers have been preserved relative to other two. The exact is reason is not known , but postulated theory says that there is preferential control of the replicative pathway over HBsAg transcription or secretion, where virion production is inhibited but secretion of subviral particles is preserved[27,28]. To determine the utility of HBsAg measurement in HBeAg-negative patients studies are needed.

The following diagram shows the comparisons of serum HbsAg levels, HBV DNA and serum ALT levels during different phase of treatment naïve chronic hepatitis B infection (figure 3).

Figure 3: HBsAg, DNA & ALT levels in different phases



HBsAg kinetics in various stages:

❖ HBeAg-positive chronic hepatitis B:

In peri-natally acquired infection, HBeAg crosses placenta and induces immune tolerance. This phase usually occurs after 10-20 years of life which is characterized by HBeAg positive,normal transminases, very high HBV DNA, and minimal histologic damage. Following which is the immune clearance phase which may leads to HBeAg seroconversion[29].

HBsAg levels vary in both immune tolerance and clearance phase but does not fluctuates unlike DNA & ALT levels. Studies have shown that the serum HBsAg levels were higher and steady in the immune tolerance phase than in the immune clearance phase . Also observed HBsAg levels were persistently high in HBeAg in immune tolerance phase[26,27]. An European study compared HBsAg levels in immune tolerance & immune clearance phase which showed the mean serum HBsAg level was 4.96 log IU/ml vs. 4.37 log IU/ ml, in immune tolerance and immune clearance phase respectively [27].

Same study showed that very high HBsAg levels(100,000 IU/ml) can be supportive of immune tolerance phase. But there is

no consensus guidelines regarding the particular cut-off to differentiate between the two phases.

- HBeAg-negative chronic hepatitis B:

The prevalence of HBeAg-negative CHB is increasing worldwide and it is important to recognize because recognizing and treating will prevent the damage to the liver. This group is classified as "inactive carrier state" and "chronic hepatitis state". Since there is wide fluctuation of serum HBV DNA levels and biochemical activity between the these two states it is difficult to diagnosis with a single determinant value and need serial measurements of ALT and HBV DNA levels[30-33].

Both the American and European guidelines recommend HBV DNA cut-off of 2000IU/ml to differentiate inactive carriers from chronic hepatitis[34,35]. But this cut-off is controversial as most of the countries are not accepting[36-38].

Quantification of HBsAg levels were studied in these groups to make it precise,definitive and supportive. Based on several longitudinal studies, they found that serum HBsAg levels are higher among active HBeAg-

negative CHB group than in inactive carriers. One study states that the mean serum HBsAg level was 3.81 log IU/ml in active CHB and in the inactive carrier state it is2.25 log IU/ml ($P<0.05$)after following for 11 years[39].

Another study states that serum HBsAg levels were significantly low in inactive (56) than in active CHB (153) with the median values of 62.12 (0.1–4068) IU/ml vs. 3029 (0.5–82,480) IU/ml, respectively ($p <0.001$)[40,41]. The exact cut-off is lacking to differentiate between the two groups. Same study said that the combination of HBsAg <1000 IU/ml and HBV DNA <2000 IU/ml rather than single variable allowed identifying inactive carriers from active CHB with a PPV 87.9% and NPV 96.7%[41]. Needs more studies to validate the particular cut –off in identifying the inactive carriers.

HBsAg kinetics with treatment response:

- **Variation with Peg-interferon treatment**

❖ HBeAg-positive patients:

Initial studies showed dramatic decrease in serum HBsAg level with interferon therapy among HBeAg positive patients and they also had sustained response (HBeAg seroconversion and HBV DNA <2000 IU/ml) 5 years post treatment . Early serological response at 12weeks (defined as low HBsAg level or greater HBsAg decline) is associated with higher rates of seroconversion and DNA suppression rates 6 months post treatment[42-44].

The following table shows various studies supporting the above evidence (table 2).

Table 2: percentage of HBeAg-positive patients with sustained virological response (SVR) as predicted by serum HBsAg at week 12 and 24 of treatment

Author	HBsAg decline	Prediction at12week		Prediction at 24 wk		Ref.
		% patient	%SVR	%patient	%SVR	
Sonneveild et al	No decline	31	3	25	8	47
Piratvisuth et al	No decline	24	18	NA	NA	48
Lau et al	<1500IUml	23	57	34	54	44
Gane et al.	<1500IU/ml	27	58	40	57	46
Chan et al.	<300IU/ml and >1og decline	NA	NA	13	75	45

These studies showed that decline in serum HBsAg level at week 12 and week 24 with peg-interferon can be used as a surrogate marker to predict sustained virological response in HBeAg-positive CHB. Also identifies the group of patients who do not respond to treatment, hence stopping rule can be applied to those non-responders. Most of the studies recommend that, absence of the decline in serum HBsAg level of >2000IU/ml at week12 stopping rule can be considered in those groups [47,48]. To recommend the above cut-off needs more studies and validation.

- HBeAg-negative patients:

HBeAg negative patients achieve undetectable DNA levels to interferon therapy but relapse post stopping. Hence monitoring HbsAg levels in those patients may add better surrogate marker for attaining sustained response. Decline in HBsAg levels do occur in HBeAg negative patients like HBeAg positive bit the response rates are comparatively low. Rijckborst V et al study states failure to decline in>2 log HBsAg and > 2log DNA levels did not respond or achieve sustained response, hence stopping rule can be considered[49].

The validation of this possible stopping rule was confirmed in the recent study done in cohorts of HBeAg-negative genotype D patients treated for either 1 or 2 years with peg-interferon alfa-2a[50].

- **Variation with nucleos(t)ide analogues:**

Following nucleoside therapy the decline in serum HBsAg levels are slow and less pronounced and does not parallel with DNA levels unlike with interferon therapy. Studies have shown that average time to HBsAg loss with NA treatment is >10years compared to DNA which is 5 years[51]. The reason for slow decline in HBsAg levels is nucleosides inhibit only the cytoplasmic pregenomic RNA but does not target the intranuclear cccDNA. On the other hand interferons acts by both immune mediated and antiviral effects[52]. It is also found that serum HBsAg levels persists even after elimination of DNA levels, with a slow progressive decline eventually[54].

- HBeAg-positive patients

Though the rate of decline is slow with NAs compare to interferon, rate of decline is comparatively rapid among HBeAg positive patients compare to HBeAg negative. A study in China conducted using 11 patients. They found that HBsAg decline of > 1 log was predictive of HBsAg. Also observed HBsAg levels <100IU/ml at the end of treatment predicted a sustained response for 2 years post stopping the drug[53].

- HBeAg-negative patients

HBsAg decline is less pronounced in HBeAg-negative patients than in HBeAg-positive .The validation of predicting the treatment response and the stopping rule is controversial in this group. Some studies showed positive impact and some had negative impact. A study done at Honkong showed patients with HBsAg decline of > 1log at 12 months could predict a sustained viral response than the patients who haad < 1 log decline in HBsAg levels [55].

However a German study showed early decline in HBsAg at 12 months did not had a sustained response[52]. In Asian study which included genotype B and C patients, they concluded that an HBsAg level of <100IU/ml with NUC's might predict lower risk of relapse and stopping can be considered[53,5,56].

But to recommend the cut-off for stopping the drug , multicentre RCT's are needed. Overall, decline in serum HBsAg is slow and does not go in parallel with HBV DNA levels during treatment with NUC's. However, a rapid decline in serum HBsAg levels, (after virological response) may identify patients who will clear HBsAg in the long-term.

CLINICAL MANIFESTATIONS :

Clinical manifestations depends upon age at infection occurs, host immunity and level of replication of virus. Infection occurring during childhood or peri-natally is asymptomatic but has high risk for chronicity where as adulthood infection will be usually symptomatic with low risk of chronicity.

Hepatitis B infection has a wide spectrum of manifestations in both acute and chronic phase.

1. Acute phase :
    - ❖ Subclinical hepatitis
    - ❖ Anicteric hepatitis
    - ❖ Icteric hepatitis
    - ❖ Acute liver failure

2. Chronic phase:
    - ❖ Inactive carrier state
    - ❖ Active Chronic hepatitis
    - ❖ Compensated cirrhosis of liver

- ❖ Decompensated cirrhosis of liver
- ❖ Hepatocellular carcinoma

3. Extrahepatic manifestations :

- ❖ Arthritis
- ❖ Dermatitis
- ❖ Glomerulonephritis
- ❖ Polyarteritis nodosa
- ❖ Cryoglubulinemia
- ❖ Polymyalgia rheumatica
- ❖ Popular acrodermatitis(Gianotti-Crosti disease)
- ❖ Serum sickness

Definitions used in hepatitis B infection:

- **Chronic hepatitis B:**

  1. HBsAg-positive 6 months
  2. Serum HBV DNA 20,000 IU/mL (10^5copies/mL),lower values 2,000- 20,000 IU/ml (10^4- 10^5 copies/mL) are often seen in HBeAg-negative chronic hepatitis B.
  3. Persistent or intermittent elevation in ALT/AST levels
  4. Liver biopsy showing chronic hepatitis with moderate or severe necro-inflammation .

- **Inactive HBsAg carrier state :**

  1. HBsAg-positive 6 months
  2. HBeAg –ve, anti-Hbe + ve
  3. Serum HBV DNA < 2,000 IU/mL
  4. Persistently normal ALT/AST levels
  5. Liver biopsy confirms absence of significant hepatitis

❖ **Resolved hepatitis B:**

1. Previous known history of acute or chronic hepatitis B or the presence of anti-HBc ± anti-HBs
2. HBsAg negative
3. Undetectable serum HBV DNA*
4. Normal ALT levels.

❖ **Acute exacerbation or flare of hepatitis B :**

Defined as intermittent elevations of aminotransferase activity to more than 10 times the upper limit of normal and more than twice the baseline value in an inactive chronic hepatitis B infection.

❖ **HBeAg clearance:**

Defined as the loss of serum HBeAg in a person who was previously HBeAg positive.

- **HBeAg seroconversion :**

  Defined as the loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative.

- **HBeAg reversion:**

  Reappearance of HBeAg in a person who was previously HBeAg negative, anti-HBe positive.

- **HBsAg seroconversion** :

  Defined as the loss of serum surface antigen(HBsAg) with appearance of anti-Hbs in the serum.

Outcome of chronic infection:

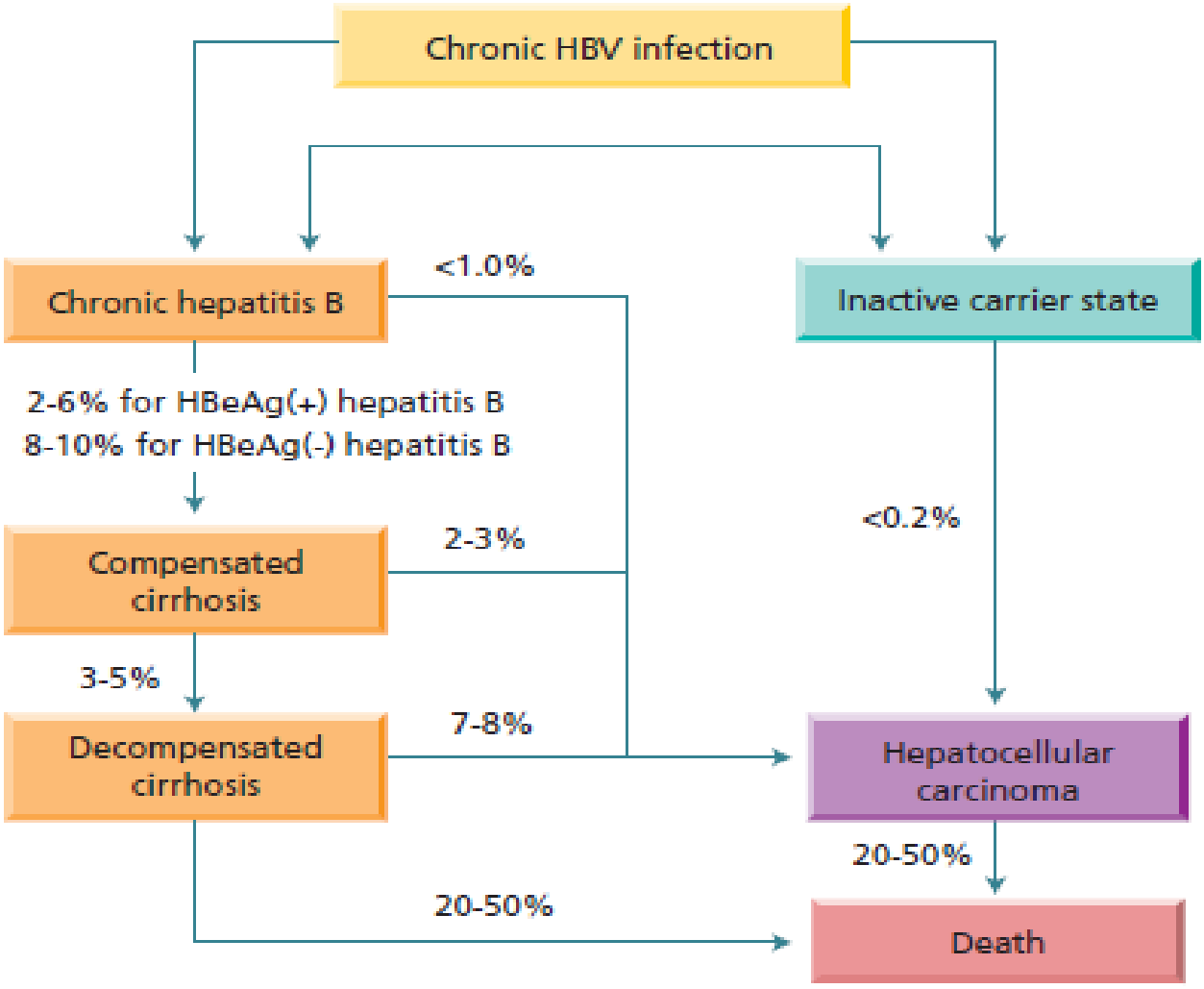


Figure 4 : course of CHB infection

Factors Associated with Progression of HBV-related Liver Disease

- Increased rates of cirrhosis

a) Host and viral risk factors

1. Older age (longer duration of infection),
2. HBV genotype C,
3. High levels of HBV DNA,
4. Habitual alcohol consumption, and
5. Co- infection with HCV(10- 15%) HDV or HIV (6-13%)

b) Enivonmental Factors

1) heavy alcohol consumption,
2) carcinogens such as aflatoxin, and,
3) smoking

- Increased rates of HCC

a) Host and viral risk factors

1) Male gender,
2) Family history of HCC,
3) Older age,
4) History of reversions from anti-HBe to HBeAg,
5) Presence of cirrhosis,
6) HBV genotype C,
7) Core promoter mutation, and
8) Coinfection with HCV.

b) Enivonmental Factors

1) heavy alcohol consumption,
2) carcinogens such as aflatoxin, and,
3) smoking

EVALUATION OF PATIENTS WITH CHRONIC HBV INFECTION:

1. History and physical examination
2. Family History of liver disease, HCC
3. Laboratory tests to assess liver disease
    i. Complete blood counts with platelets
    ii. Liver function test,
    iii. Prothrombin time
    iv. Renal function test
4. Tests for HBV replication : HBeAg/anti-HBe, HBV DNA(RT- PCR)
5. Tests to rule out viral co-infections: anti-HCV and anti-HIV in those at risk
6. Tests to screen for HCC–AFP and ultrasound liver at baseline and every 6months in high risk patients.
7. Consider liver biopsy to grade and stage liver disease
    - for patients who meet criteria for chronic hepatitis

APPROVED THERAPY:

Currently, seven therapeutic agents have been approved for the treatment of adults with chronic hepatitis B in the United States. The use of interferons in the management of CHB is restricted with the wide availability nucleotide analogues. The duration of treatment varies with the type of therapy and stage of the disease. The interferons are used only for 52 weeks and their response also varies with the type of genotype, where as nucleoside analogues duration is not fixed unlike interferons. Duration of therapy with nucleotides varies with the stage of disease.

- Interferon (IFN) — conventional and pegylated.

- Five nucleos(t)ide analogues under three groups.

  L –nucleosides – Lamivudine and Telbivudine;

  Acyclic nucleoside – Adefovir dipivoxil

  Tenofovir disoproxil fumarate; and

  Deoxyguanosine analogues – Entecavir

Table 3 :

Drugs	**Advantages**	**Disadvantages**
Interferons	• Finite therapy • Durable off treatment response • 5 -8% of HbsAg loss	• Iv injections • Side effects • Expensive • Low response rate in pts with high viremia
Nucleosides	• Negligible side effects • Potent inhibition of viral replication. • Less expensive	• Drug resistance • Therapy duration? • Lowest rate of HbsAg disappearance

- Factors to Consider in Initiating anti-HBV Therapy

The factors to be considered before initiating the therapy are based on the following

i. HBV DNA levels

ii. ALT levels

iii. HBeAg status

iv. Cirrhosis vs no cirrhoisis

v. Compensated cirrhosis vs decompensated

vi. Others

- Pregnancy state to prevent vertical transmission
- Prior to chemotherapy
- HIV positive state

Treatment recommendations :

Table 4 : AASLD recommendations

HBeAg	HBV DNA (PCR; IU/mL)	ALT	Management
+	>20,000	≤2 x ULN	Observe; biopsy if >40 y, ALT high-normal to 2 x ULN, or family history of HCC; treat if moderate/severe inflammation, significant fibrosis, or ALT becomes elevated
+	>20,000	>2 x ULN	Treat immediately if icteric/clinical decompensation; otherwise, observe 3–6 mo, consider biopsy; treat if no HBeAg loss
–	>20,000	>2 x ULN	Treat
–	>2000	1–>2 x ULN	Consider biopsy; treat if moderate/severe inflammation or significant fibrosis
–	≤2000	≤ULN	Observe; treat if HBV DNA or ALT become elevated
+/–	Detectable	Cirrhosis	If compensated, treat if HBV DNA >2000 IU/mL or ALT elevated; if decompensated, refer for liver transplant
+/–	Undetectable	Cirrhosis	If compensated, observe; if decompensated, refer for liver transplant

TREATMENT END POINTS:

- Interferon regimes :
  - HBeAg-positive chronic hepatitis B

    -16 weeks for standard IFN

    -48 weeks for pegIFN-. (I)

  - HBeAg-negative chronic hepatitis B

    - 48 weeks for both standard and pegIFN- (II-3)

- Duration of nucleoside analogue treatment:
  - **HBeAg-positive chronic hepatitis B**:

    - Treatment should be continued until the patient has achieved HBeAg seroconversion and undetectable serum HBV DNA and completed at least 6 months of additional treatment after appearance of anti-HBe. (I)

- Close monitoring for relapse is needed after withdrawal of treatment.

❖ **HBeAg-negative chronic hepatitis B** :

- Treatment should be continued until the patient has achieved HBsAg clearance. (I)

❖ **Compensated cirrhosis** :

Ideally long-term treatment is advised.

a) <u>HBeAg-positive patients</u>

Till confirmed HBeAg seroconversion and have completed at least 6 months of consolidation therapy.

b) <u>HBeAg-negative patients</u>

Till confirmed HBsAg clearance. (II-3)

- Close monitoring for viral relapse and hepatitis flare is mandatory if treatment is stopped. (II-3).

❖ ***Decompensated cirrhosis and recurrent hepatitis B post–liver transplantation*:**

Life-long treatment is recommended. (II-3)

MATERIALS AND METHODS

The study population included all the patients of chronic hepatitis B fulfilling the inclusion criteria who attended Department of Digestive Health and Diseases, Government Peripheral Hospital, Anna Nagar, Chennai-600 102.

The period of study is from January 2014 to January 2015. Patients were included in the study after obtaining their willingness to undergo necessary investigations. Informed written consent was obtained from the study participants before enrollment.

Ethical Committee approval was obtained for performing the study.

Inclusion criteria :

1. Chronic hepatitis B infection

2. HBV related compensated cirrhosis of liver

3. HBV related decompensated cirrhosis of liver

Exclusion criteria :

1. Acute hepatitis B
2. Co infection with HCV
3. Co infection with HIV

Methodology

All the patients satisfying the inclusion and exclusion criteria were enrolled in to the study.

Follow up :

Liver function tests, HBsAg quantification and HBV-DNA levels were done for every 12 weeks for 1 year .

- The diagnosis of chronic hepatitis B is made on the basis of duration of HBsAg status(HbsAg positive for more than 6 months).
- The diagnosis of chronic liver disease is made on the basis of evidence of portal hypertension and cirrhosis in ultrasonography and presence of varices in upper gastrointestinal endoscopy.

Clinical evaluation:

- Detailed history regarding present and past history of jaundice, ascites, gastrointestinal bleeding, pedal edema, hepatic encephalopathy, blood transfusion, surgeries, extra marital sexual exposure, alcohol intake, associated co-morbidities, etc. were noted.

- Clinical examination was done to check for the evidence of chronic liver disease such as jaundice, spider angioma, dupuytrens contracture, palmar erythema, gynaecomastia, ascites, splenomegaly, caputmedusae, asterixis.

Laboratory investigations:

Blood investigations like hemoglobin, WBC count, platelet count, prothrombin time, INR, S.bilirubin, T.protein, albumin, alanine aminotransferase, aspartate aminotransferases, HBSAg, Anti HCV, HIV, urea, creatinine, USG abdomen, Gastroscopy, liver biopsy if necessary. Child Turcotte Pugh score and MELD score were calculated using the various parameters.

Baseline measurements: HBsAg quantification, HBV DNA levels.

Treatment :

Those who fulfilled the criteria for treatment recommendation were started on the available drugs

- Tenofovir
- Lamivudine(Children)

STATISTICS:

Statistical analyses of these data was done using Receiver operating curve and Pearson co-efficient equation .

Any statistical difference was considered significant at P< 0.05.

p.value 0.000 to 0.010 is highly significant.

p.value 0.011 to 0.050 is significant.

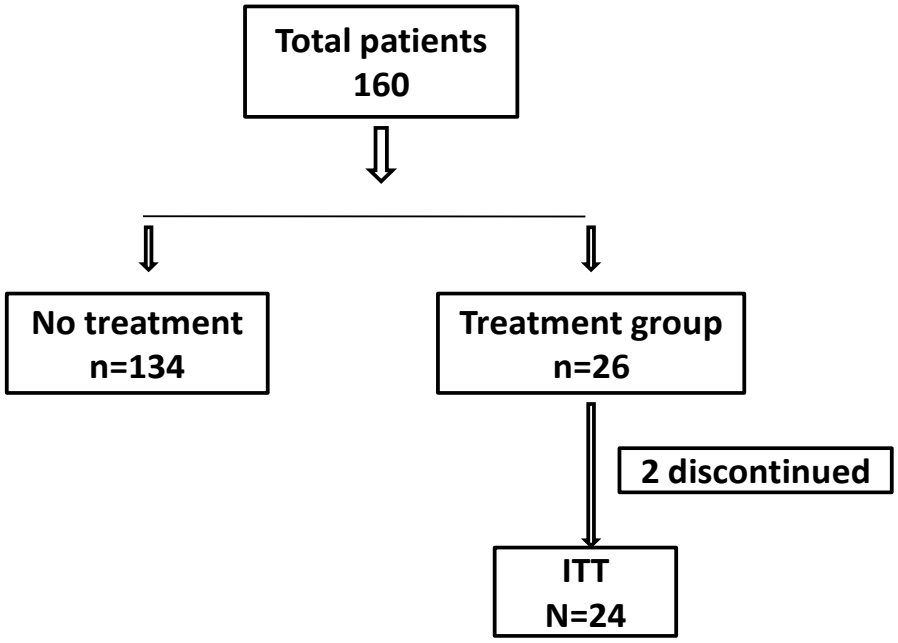
p.value 0.051 to 1.000 is not significant.

RESULTS

Total of 160 patients were included in my study, of which 26 are in the treatment group. The results are as follows

FLOW CHART OF THE STUDY:

Figure 5 :



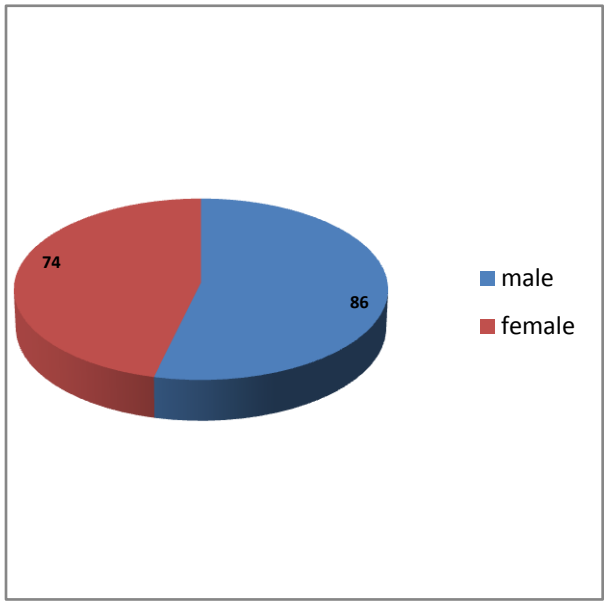
ITT: Intention to treat

Sex distribution :

Table 5: sex distribution

sex	Male	Female	Total
No .s	86(53.75%)	74(46.25%)	160

Figure 5: pie chart showing sex distribution



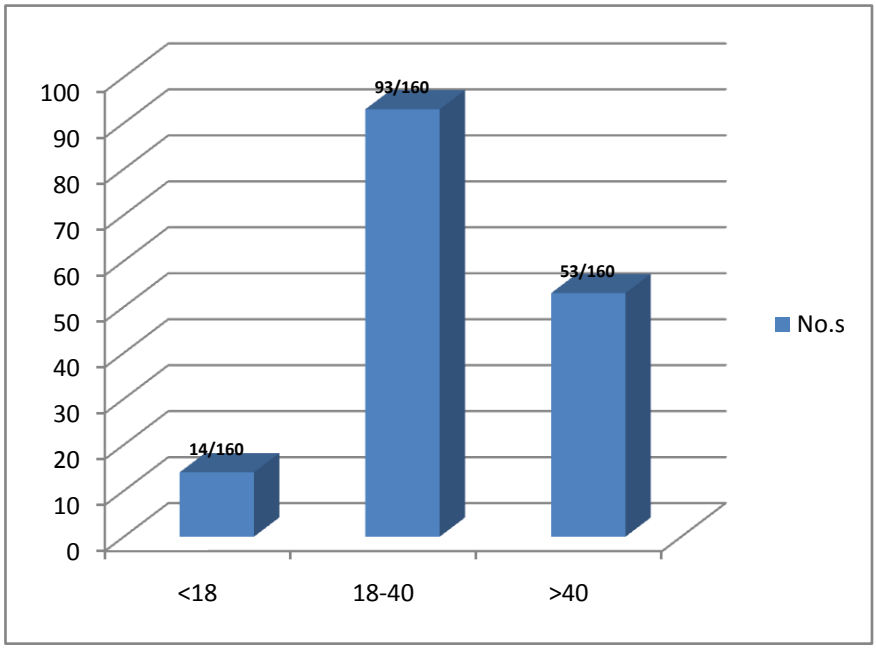
In our study we found that males are more than females but there is no significant sex predilection. This may be explained by the predominant route of transmission (vertical transmission) in intermediate prevalent regions like India.

Age distribution

Table 6 : showing age distribution

Age in yrs	<18	18-40	>40	Total
No .s	14 (8.75%)	93(58.12%)	53(33.1%)	160

Figure 6 : Bar diagram showing age distribution



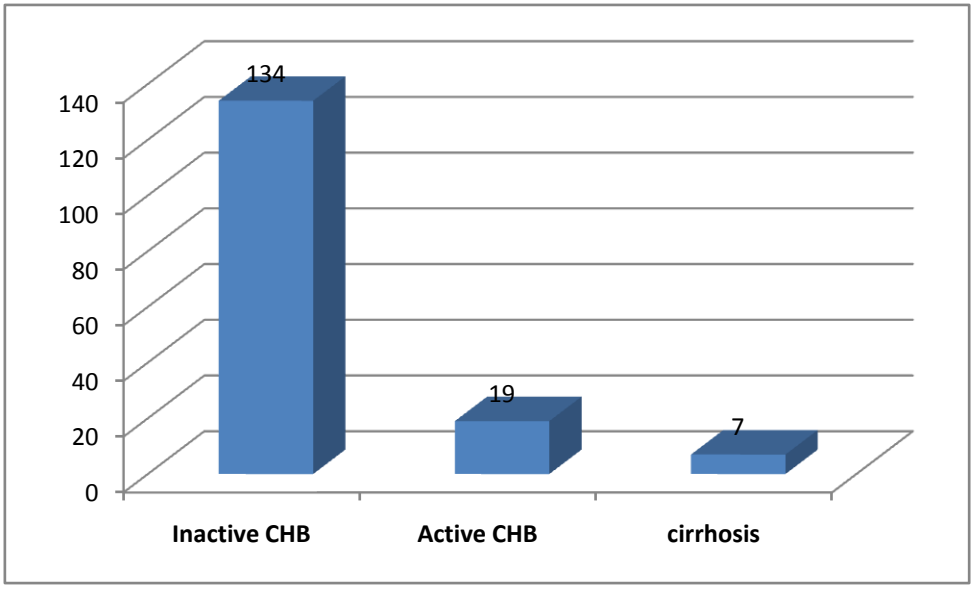
Hepatitis B infection can occur at any age group. In my study, 8.75% were in the age group less than 18 years , 58% were in the age group of 18-40 years and 33% (53/160) were above 40 years, of which 11(53) were above 60 years of age group . This shows that young adults are most common affected age group, indicating that infection has occurred either in infancy or childhood. This can be explained by the intermediate prevalent region.

Stage wise distribution of CHB:

Table 7 : showing various stages of CHB

Staging	**Inactive CHB**	**Active CHB**	**Cirrhosis**	**Total**
No.s	134(83.75%)	19(11.87%)	7(4.37%)	160

Figure 7 : Bar diagram showing Phasic distribution of CHB



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Among chronic hepatitis B infection, 83.75% were in inactive chronic hepatitis B phase(asymptomatic carriers).11.87% were in active chronic hepatitis B phase and 4.37% are cirrhotics. Our study found that asymptomatic carrier state is the commonest mode of presentation followed by active chronic hepatitis infection and the least is cirrhosis of liver. Hence this study correlates well with the available literature.

Correlation of qHBsAg levels with HBV DNA levels:

Correlation between HBeAg positive & HBeAg negative group

Table 8 : showing qHBsAg & DNA levels in e-antigen positive and e-negative groups

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In our study we found, serum HBsAg levels are higher in HBeAg positive group than HBeAg negative group with a mean of 4.25logIU/ml and 2.81logIU/ml respectively. Also HBsAg levels significantly correlates with HBVDNA levels in HBeAg positive group with a P value of <0.001. But serum qHbsAg levels did not show significant correlation with HBV DNA levels in HBeAg negative patients.

	HBV DNA (logIU/ml)	**qHBsAg (logIU/ml)**	**P value**
HBeAg positive	6.51	4.25	<0.001
HBeAg negative	2.5	2.81	0.147

The similar observations were also seen in other studies . A study by Jeyamani et al [57] found that serum HBsAg levels were higher in HBeAg positive patients with a mean of 4.52logIU/ml and significantly correlated well with DNA levels but in HBeAg negative group ,the serum HbsAg levels were lower with a mean 3.2logIU/ml and did not correlate with HBV DNA levels. A study by Jaroszewicz also found the similar results with higher HBsAg levels in HBeAg positive group than in HBeAg negative group[7].

Subgroup analysis in HBeAg positive group.

It is difficult to differentiate between immune-tolerance and immune-clearance phase with ALT & HBV DNA levels. But serum HBsAg levels were higher in immune tolerant phase than in immune-clearance phase which was observed in a study by Jaymani et al [57].

The following table shows the analysis of qHBsAg levels in these two groups in our study.

Table 9 : qHBsAg and DNA levels in Immune -tolerant & Immune-clearance patients

	Immunetolerant	**Immuneclearance**	**P value**
ALT	25 (19-36)	76 (42-204)	0.61
DNA (logIU/ml)	6.79	6.08	0.79
qHBsAg(logIU/ml)	4.59	3.73	0.038

We observed that serum HBV DNA levels and ALT levels did not show significant difference among immune-tolerant and immune-clearance phase. So

these variables cannot differentiate immune-tolerant from immune-clearance phase (P= NS), where as serum qHBsAg levels were higher in immune-tolerant phase than immune-clearance phase with mean value of 4.59logIU/ml in immune-tolerant phase and 3.74logIU/ml in immune-clearance phase. So qHBsAg levels can differentiates the two phases with a significant P value of 0.038.

Two cross-sectional studies from Europe and Asia also found the higher serum HBsAg levels in immune-tolerant phase with a mean value of 4.96logIU/ml in immune tolerant and 4.37 logIU/ml in immune-clearance phase[27]. Our study correlates with the other studies.

qHBsAg levels in Inactive carriers Vs Active CHB:

Table 10 : showing qHBsAg levels in inactive & Active CHB

	Inactive carrier (DNA<2000Iu/ml)	**Active infection (DNA>2000IU/ml)**	**P value**
qHBsAg	2.64	4.20	0.02

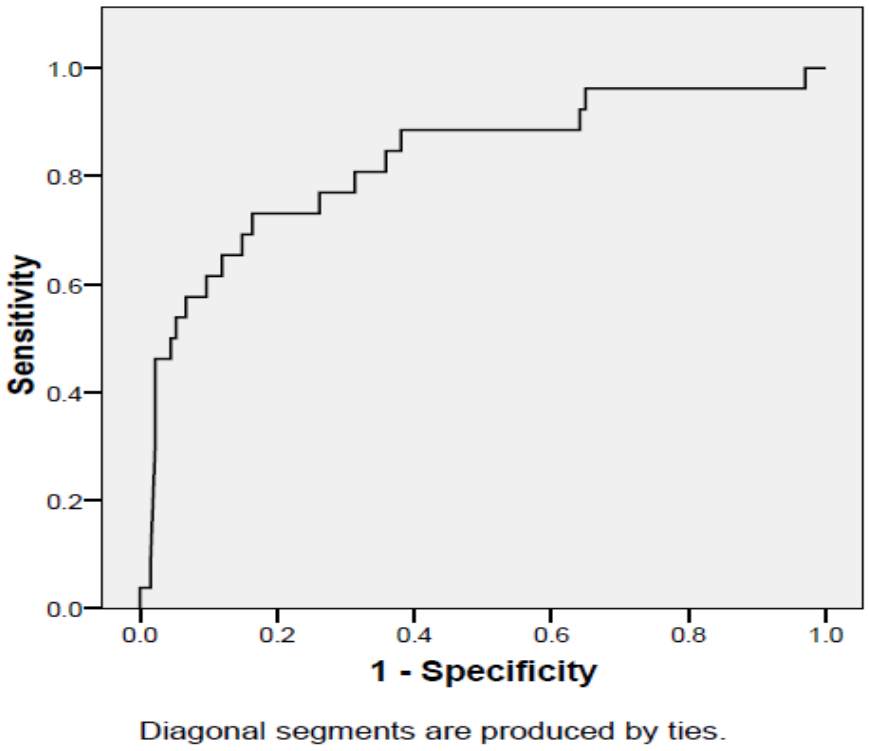
Serum HbsAg levels are higher in active chronic hepatitis B group than inactive carriers with a values of 4.20logIU/ml and 2.64logIU/ml respectively (P=0.002). Hence higher values indicates active disease .

The same observations were found in another Indian study by Jeyamani et al, stating that higher values are seen in active CHB than inactive carriers[57].

Diagnostic utility of qHBsAg in HbeAg positivity:

When Reciever Operating characteristics Curve(ROC) was drawn to predict the status of HBeAg positivity by serum HBsAg levels, the area under the curve is 0.82 with a significant P value of <0.001 (95% confidence interval is 0.73 – 0.92). The curve follows as.

Figure 7 : ROC curve depicting qHBsAg in HBeAg positive state



From the above ROC curve, serum HBsAg level of 3.01logIU/ml indicates high chances of replicative state with 96% sensitivity and 76% specificty.

Where as HBeAg negative ROC curve showed low diagnostic utility. Diagnostic utility of HBsAg levels in HBeAg positive group showed positive results in other studies using ROC curve by Jeyamani et al [57].

CORRELATION OF qHBSAG LEVELS IN THE TREATMENT GROUP :

Among 160 patients only 24 were included in treatment group . All 24 were treated with Nucleotide analogues(Tenofovir). Post treatment HbsAg levels and DNA levels are monitored every 12 weeks.

Follow up period was 36 weeks with the treatment.

There results are as follows.

HBeAg positive group :

Table 11 : correlation of qHBsAg levels with DNA

Variables	**Baseline** (logIU/ml)	**12 weeks** (logIU/ml)	**36 weeks** (logIU/ml)	**P value**
qHBsAg	4.49(3.8-5.0)	4.35	4.13(3.0-4.8)	NS
DNA	6.97(3.5-8.0)	4.59	4.08(2.8-4.9)	0.002

With the NUC's therapy the decrease in qHBsAg levels are very slow and showed less than 1log drop at 12 weeks from baseline, the response at 36 weeks

also did not show significant reduction in serum HBsAg levels(P=NS). On the other hand DNA levels showed >1log drop from baseline and had good response at 36 weeks(P=0.002).

The data from the studies by Cai et al, Jaroszewicz et al, borgniet et al. suggests that HBsAg reduction with NUS's are slow and less pronounced than interferon treatment, despite significant drop in HBV DNA levels[52-54]. Our study also supports the existing data.

HBeAg negative group :

Table 12 : correlation of qHBsAg with DNA levels

Variables	**Baseline** (logIU/ml)	**12 weeks** (logIU/ml)	**36 weeks** (logIU/ml)	**P value**
qHBsAg	3.87(2.5-4.98)	3.69	3.03	o.87
DNA	5.56(3.7-8.0)	5.06	3.56(0.7-4.0)	0.001

In HBeAg negative group, decline in serum HBsAg levels follow the same pattern as in HBeAg positive group i.e slower rate without significant decline at 36 weeks. Though the HBVDNA decline is also slow in HBeAg negative group compared to HBeAg positive group, but there is significant reduction (P=0.001) at 36 weeks when compared to HBsAg levels . Hence serum HBsAg levels does not go in parallel with DNA levels.

The results in our study are similar to other studies by Jaroszewicz et al [52] and Cai et al[53].

Table 13 : CHB patients with undetectable DNA but detectable qHBsAg

Variables	**qHbsAg** (n=160)	**DNA** (n=160)
No .s	18	0

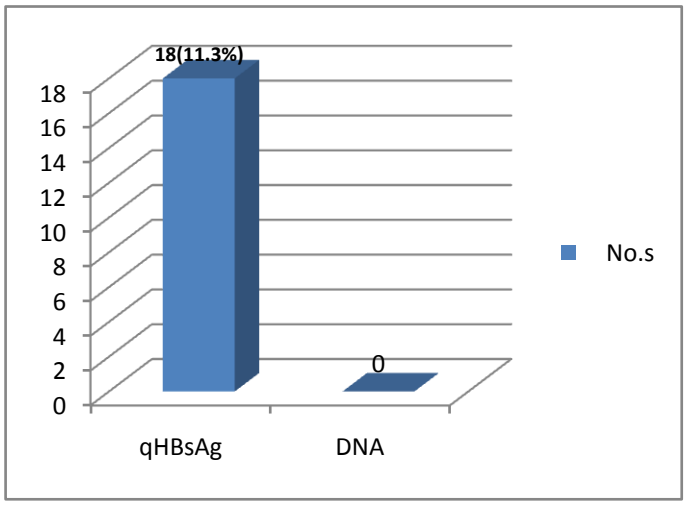


Figure 8: Bar diagram showing % of qHBsAg in undetectable DNA

Among 160 patients in our study, undetectable DNA levels found in 18(11.3%) patients but serum HBsAg levels were detected in these 18 patients. In those 15/18 were in inactive carrier stage, 2/18 were in active chronic hepatitis B and 1/18 in cirrhotic group. The latter two groups were on NUC's therapy.

This indicates that serum HBsAg levels take a longer course to become undetectable after the elimination of DNA levels from serum.

As supported by Borgniet et al, the average time taken for HBsAg loss after undetectable HBV DNA was around 30 months [54].

DISCUSSION

HBsAg levels reflects the true cccDNA levels rather than serum HBV DNA levels. Hence HbsAg quantification (qHBsAg) can serve as a biomarker for prognosis and the treatment response in CHB. Though its importance was recognized in the past, its usefullness and interest has captured the Hepatologist recently. The reason being is it has shown strong positive correlation with intrahepatic cccDNA levels, so it has a ability to predict spontaneous HBsAg clearance and e-antigen seroconversion on antiviral therapy[58]. The other important applications of qHBsAg levels are , low levels at baseline predicts interferon induced HBeAg serocnversion and ability to stratify the risk for the development of Hepatocellular carcinoma(HCC) in CHB. A study by Lin et el. have shown that qHBsAg>1000IU/ml & DNA<2000IU/ml is associated with high risk of HCC[59].

Ours is a prospective study done to evaluate the role of qHBsAg levels in comparing with HBV DNA levels in the natural course of treatment naïve CHB infection . Our study also monitored the qHBsAg levels in patients on NUC's to look for the response in predicting the sustained response in compared with HBV DNA levels.

Clinical characteristics :

Our study showed no sex predeliction and the common age of presentation was between 18-40years , which correlate with the available data. Among 160 patients, majority were in inactive carrier state (83.75%). The rest being active CHB (11.87%) and cirrhosis of liver in 4.37%. The commonest presentation in our study is asymptomatic i.e incidentally detected on regular master check up or screening of blood donors.

HBsAg kinetics in treatment naïve CHB:

On evaluating qHBsAg levels in CHB patients, we found that qHBsAg levels were higher in HBeAg positive group with a median value of 4.25logIU/ml. This correlate significantly with DNA levels (P = <0.001). These findings are consistent with studies by Jayamani et al[57] and other Asian & European countries [24,28]. These studies also found higher and significant qHBsAg in HbeAg positive patients.

On the otherhand qHBsAg levels are much lower in HBeAg negative patients with a median of 2.8logIU/ml and does not correlate with

DNA levels unlike HBeAg positive . An Italian study by Brunneto et al[28] found the similar results.

A subgroup analysis was done to differentiate between inactive chronic hepatits B infection from active chronic hepatitis B. This study found that the serum qHBsAg levels are higher in active chronic hepatitis B patients with a median of 4.20logIU/ml when compared to inactive chronic hepatitis B patients with a median value of 2.64logIU/ml, which is statistically significant (P=0.002). Hence the higher value clearly differentiates inactive CHB from active CHB patients.

A longitudinal study from Hongkong also found that higher qHbsAg levels in active disease and significatnly differentiates between active and inactive CHB with a median values of 2.98logIU/ml vs 2.24logIU/ml (P = 0.05) respectively[24]. Another Itlalian study by Brunetto et al found that serum HBsAg levels are significatly lower in inactive CHB than active CHB[28].

In CHB , it is difficult to differentiate immunotolerant phase from immunoclarence phase with ALT & DNA levels as

these levels will be fluctuating throught the course. Though the American and European guidelines uses DNA cut off of 2000IU/ml to differentiate the two , controversy still exists in many countries. Hence we studied the role of qHBsAg levels in differentiating immunotolerant from immunoclearance phase. We found that serum qHBsAg levels were significantly higher inimmunotolerant phase with a median of 4.59logIU/ml when compared to immunoclearance phase with a median of 3.73logIU/ml ($p = 0.038$).

Studies from Europe and Hongkong also found a similar results of higher values in immunotolerant phase than immunoclearnace phase with a median of 5logIU/ml and 4.9logIUml respectively[5]. These studies also concluded that serum qHBsAg value of more than100000IU/ml significantly differentiated between immune-tolerant and immune-clearance phase. But we did not find the particular single value in differentiating the two.

We also found that single point estimation of serum qHBsAg using ROC curve with a value of 3.1logIU/ml indicates high chances of predicting replicative state with 96% senstivity and 76% specificity. Our study is supported by another indian study by Jeyamani et al which also showed the similar results[57].

qHBsAg kinetics with NUC's:

Data suggests that fall in serum qHBsAg levels are slower and less pronounced with NUC's when compared to Interferon therapy. Serum qHBsAg fall does not fall in parallel with DNA levels with NUC's.

Our study showed decline in qHBsAg levels with NUC's but the rate of fall is slower when compared to DNA levels among both HBeAg positive and HBeAg negative patients. At 12 weeks with NUC's therapy , DNA levels showed >1logIU/ml reduction from the baseline but serum qHBsAg levels showed <1logIU/ml reduction from baseline. So serum qHBsAg levels did not correlate well with DNA levels on patients with NUC' therapy.

Brunetto et al. and Chan et al. found that drop in serum qHBsAg levels of more than 1logIU/ml from baseline would predict the HbsAg seroclearance on follow up of 34± 23 months. Also found that fall in HbsAg level can predict the stopping the therapy [41,55]. But our study followed only for 36 weeks, hence prediction of sustained response was not able to conclude based on the available data.

We also found that in around 11.25% (18/160) serum qHBsAg levels were detected despite undetectable DNA levels. The reason being qHBsAg levels takes long time to disappear from serum. Same findings were also observed in another study by Borgniet et al[54]. This indicates that serum qHBsAg levels might be better marker in predicting seroclearance and can guide in stopping the therapy. Further studies with longer duration are needed to conclude.

CONCLUSION

1. High serum qHBsAg levels has a good correlation with HBV DNA levels in HBeAg positive than HBeAg negative patients.
2. Hence single point estimation of qHBsAg levels can predict replicative state and can act as a surrogate marker for the replicative state.
3. Higher qHBsAg levels also differentiates inactive CHB from active CHB and can replace HBV DNA levels in differentiatin the two. Estimation of qHBsAg is easy and cost effective.
4. Serum qHBsAg levels decline slowly with NUC's than DNA levels and decrease in serum qHBsAg levels does not correlate with decrease in HBV DNA levels.

LIMITATIONS

1. Single centre study
2. Genotype assesment and qHbsAg kinetics based on genotypes were not assessed
3. Treatment was followed only for short duration.

SUGGESTIONS

1. Need multicentre study
2. qHBsAg kinetics based on genotypes
3. Longer duration of follow up
4. Standardising the partcicular cutt-off of qHbsAg levels for stopping rule during therapy.

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Role of serum HBsAg quantification in treatment of chronic hepatitis B infection.

Proforma

Name : Age/Sex: IP No:

Complaints :

Past history:

Family history :

Personal history : alcohol : smoking:

Others :

Examination :

GPE: pallor/icterus/cyanosis/clubbing/edema/lymphnodes

Pulse : B.P

Abdomen :

R.S: CVS:

CNS:

Investigations :

Hb%: TC: ESR: Plt:

LFT: TB: DB/IB: OT/PT: SAP:
Protein/albumin/globulin:

RBS : FBS/PPBS:

PT/INR:

Blood urea: S.Creatinine:

HBsAg: HBeAg/antiHBeAg:

IgMantiHBc:

	Baseline	6 mnths	12mnths
qHBsAg			
HBV-DNA			

Anti HCV: HIV:

USG abdomen: Chest X-ray:

OGD :

Antiviral drugs:	name :	duration

CTP Score: MELD :

Diagnosis :

Ac.Hepatitis	CHB	Compen.CLD	DCLD

S.No	Age	Sex	DDHD NO	liver function test						HBeAg	DNA(IU/L)	QHBsAg (IU/L)	USG ABDOMEN	LIVER BIOPSY	OGD	FIBROSCAN
				bilirubin	SGOT	SGPT	PROTEIN	ALBUMIN	GLOBULIN							
1	33	M	1204/14	1	32	18	7.1	4	3.1	negative	3330000	12374.5	normal			
2	33	F		0.8	24	22	6.9	3.9	3	negative	306/940	4540.14/17354.74	normal			
3	52	F		1.4	41	28	7.4	4.4	3	negative	9.62	4197.72	fatty liver			
4	49	F		1	20	19	6.8	3.6	3.2	negative	153	2756.62	normal			
5	25	F		0.9	30	20	6.9	4.1	2.8	negative	43.9/<6	5237.33/10510.83	normal			
6	31	f		1.1	33	23	7.3	4.3	3	negative	2000	11359.04	normal			
7	57	M		0.7	46	38	7.4	5.4	2	negative	196	10779.94	fatty liver			
8	37	F		1.6	50	36	7.6	4.4	3.2	negative	361	6792.46	normal			
9	2	M	31/14	0.7	54	50	7.2	3.8	3.14	**reactive**	>110,000000	>100000	normal			
10	45	F	322/14	0.37	27	25	6.8	3.7	3.1	negative	<6	978.64	normal			
11	27	F		1.2	22	32	7.2	4.2	3	negative	6.18	18675.8	normal			
12	35	M		1.6	56	48	7.9	3.6	4.3	negative	1.83	2583.43	normal			
13	22	F		1.1	54	90	7.2	4.2	3	**reactive**	>110,000000/80,600	71116.85/63078.93	normal			
14	48	F		1	22	30	7	4.1	2.9	negative	363	19435.73	fatty liver			
15	58	M		1.2	33	30	6.4	3.8	2.4	negative	1250	1323.78	fatty liver			
16	22	M		0.8	42	38	6.8	4	2.8	negative	16.3	20796.59	normal			
17	38	M	5114/13	0.6	15	19	7	4.7	2.3	negative	124,000	2308.7	normal			
18	32	M		1.2	22	32	7.2	4.2	3	negative	39.8	5276	normal			
19	23	M	227/14	1.2	27	14	7.4	3.6	3.8	negative	902	2371.77	normal			
20	34	M	213/14	1.6	56	38	7.6	4.4	3.2	**reactive**	3,81,00	33961.75	fatty liver			
21	23	M		0.5	27	25	7	4		negative	6.66	1672.93	normal			
22	33	F	136/14	1.2	27	14	7.4	3.6	2.8	negative	563	1444.62	normal			
23	72	F		1.1	58	72	7.2	4.3	3	**reactive**	19,700,000	5657.79	fatty liver			
24	50	F	1169/14	0.7	108	83	7.4	2	5.4	**reactive**	>110000000/1060000/145	7712.74/939.82	normal			
25	54	M		0.65	24	20	7	4		negative	undetected	939.82	normal			
26	21	M		2	46	21	7.2	4.8		**reactive**	>1100000001	<11791.82	fatty liver			
27	28	M		0.55	27	25	6.8			negative	361	9137-57	fatty liver			
28	18	M	206/13	1	25	37	8	4.2	3.8	negative	48000000	15341.81	normal			
29	32	M		1	158	83	7.6	4	2.6	**reactive**	4329	18317.08	normal			
30	43	M	0006/14	1.6	23	91	8.1	5.1	3	negative	5030	121.78	cld			
31	37	F		1	19	22	7.7	4.7		negative	nill	0.03	normal			
32	63	M	2066/14	0.9	91	62	6	2.5	3.5	negative	81,300	2062.28	cld			
33	21	F	573/14	1.1	44	31	7.2	4.1	3.1	negative	1850	7625.83	fatty liver			5.7pka
34	18	M	017/06/14	2.3	46	21	7.2	4.5	2.7	**reactive**	>110000000,4640	22769.70, 33043.4	normal	chronic active hapatitis		
35	12	F	343/14	1	26	12	6.7	4.3	2.4	**reactive**	42700, undetected	4433.84,3079.31	normal			
36	51	M	014/7/14	0.9	42	28	6.6	3.8	2.8	negative	40.1	1870.55	normal			
37	69	M	3113/14	1	24	22	6	3.5	2.5	negative	559	414.9	normal			
38	23	M	1197/14	0.7	19	26	6.8	3.8	3	negative	551	19112.24	normal			
39	29	M	2121/14	0.95	21	38	7.2	4.5	2.7	negative	687	75.75	normal			
40	65	M		1	32	30	6	4	2	negative	8.89	64.11	normal			
41	39	M		1.3	34	30	6.7	4.3	2.4	negative	87.9	2051.8	normal			
42	64	M	3960/12	1.6	128	138	7.6	3.6	4	**reactive**	15,000000, 1040	66000, 1385.77	CLD		GIIVx	
43	24	M		1.2	27	25	6	3.5	2.5	negative	14.5	1081.7	normal			
44	42	M		1.1	34	28	6.4	4.4	2	negative	1140	2996.78	normal			
45	17	F		1.8	46	44	7.2	4.5	2.7	**reactive**	27000000	27000	normal			
46	42	M		0.8	50	41	6.8	4.4	2.4	negative	736	4751.55	normal			
47	36	F		1	32	30	7.1	4.1	3	negative	undetected	1632.61	normal			
48	39	M		0.85	26	34	7.2	3.8	3.4	negative	1650	1167.92	normal			
49	48	M	615/12	1.8	50	23	8.1	3.6	4.5	negative	95.5	367.77	CLD			
50	22	F	6101/12	0.9	42	24	7.1	4.6	3	negative	<6	1130.77	normal			
51	27	F	017/7/14	4.3	42	24	7.6	2.9	4.2	negative	<6	189.9	CLD			
52	27	F	1177/09	0.9	42	28	5	2	3	negative	560	21780.62	CLD			
53	67	M	168/14	0.9	26	34	7.2	3.8	3.4	negative	undetected	0.02	normal			
54	43	M		1.1	26	24	6.7	4.5	2.2	negative	649	1282.59	normal			
55	20	F		1.2	48	54	6.9	3.6	3.3	negative	70.7	11635	fatty liver			
56	24	F		1.5	52	42	6.8	4.4	2.4	negative	833000	1588.25	fatty liver			
57	51	F		0.8	32	22	6.6	4.6	2.4	negative	undetected	1.83	normal			

58	19	F		0.65	19	21	7	4.1	2.9		negative	<6.0	538.64	normal						
59	27	F		0.5	22	18	6.9	3.9	3		negative	202	0.03	normal						
60	49	M		1	32	24	6	3.5	2.5		negative	<6	1808.04	normal						
61	15	M	7665/13	0.2	26	34	7.1	4.2	2.9		**reactive**	>1100000, 131000	>100000, 100000	normal						
62	6	F	7652/13	0.4	49	47	6.9	4.9	2		**reactive**	110000, 25700	>100000, 73492	normal						
63	29	F	3594/13	0.8	52	34	7	4	3		negative	29.2	2765.9	normal						
64	59	F		1.1	28	34	6.6	4.2	2.4		negative	undetected	0.8	normal						
65	29	M		1.2	47	42	7.2	3.7	3.5		negative	64.5	32657.22	normal		mild hepatitis				
66	53	M		1	60	44	6.8	4.8	2		negative	>110000000	6706.75	fatty liver						
67	29	M	934/14	1	34	25	8.2	4.8	3.4		negative	68.9	2315	normal						
68	27	M	1500/10	2.1	30	24	7.7	4.5	3.2		**reactive**	111	14977.6	normal						
69	65	M	1878/14	6.9	650	570	6	4.2	2.8		**reactive**	3730000	96719.9	fatty liver						
70	34	M	2374/14	2.1	192	40	7.3	3.2	4.1		negative	99	3214	CLD						
71	19	F	2561/14	0.7	27	20	7.1	4.6	2.5		negative	8.76	21165.07	normal						
72	47	M	2559/14	0.6	23	17	6.8	4	2.8		negative	70100	3989	normal						
73	42	M	2563/14	0.9	29	33	7	4	3		negative	5432	9762	fatty liver						
74	31	F	2579/14	0.8	14	89	6.4	4.2	2.7		negative	1800	12413.73	normal						
75	36	M		1	30	26	7.2	4.2	3		negative	42.3	1093	normal						
76	34	F	2615/14	1.2	38	40	6.9	4	2.9		negative	3220	3109.21	normal						
77	14	M	2386	0.5	48	30	7.4	4.4	3.4		**reactive**	>110000000	1000000	fatty liver						
78	26	F	2087/14	0.4	48	34	6.6	3.6	2		negative	541	3000	normal						
79	24	F	2649/14	0.9	33	23	7.7	4.5	3.2		negative	6.9	3608.26	normal						
80	50	F	4174/12	1.6	52	29	7.7	3.5	4.2		negative	108	1207	normal						
81	33	F	2385/14	0.3	32	36	6.9	4.6	2.3		negative	319	23264	normal						
82	39	F	2227/14	0.5	52	54	6.4	3.2	3.2		negative	109	1631	fatty liver						
83	35	M		1.1	41	38	6.4	4.2	2.2		**reactive**	529	1499.78	fatty liver						
84	37	M	1520/14	0.9	37	35	7.9	5.3	2.6		negative	<6	273.22	fatty liver						
85	33	M		1.6	36	29	7.2	4	3.2		negative	224	52458	fatty liver						
86	25	F		1.1	40	45	7	4.1	2.9		negative	<6	3135	normal						
87	27	F	2223/14	0.46	21	16	6.4	3.4	3		negative	184000	11255.59	normal						
88	27	F	1914/14	0.9	39	33	6.9	3.9	3		negative	undetected	141.69	normal						
89	32	F		0.89	24	29	6.7	4.7	2		negative	undetected	0.9	normal						
90	72	M		1.2	31	29	6.4	3.2	3.2		negative	<6	35.02	fatty liver						
91	30	F		0.9	39	28	7.7	4.7	3		negative	<6	2636.7	normal						
92	15	M		1	21	19	6.2	3.6	2.6		**reactive**	>110000000	100000	normal						
93	18	F	2334/14	0.8	21	57	6.6	4	2.6		**reactive**	>110000000	100000	normal						
94	21	F	030/6/14	1	20	36	6.9	4	2.9		negative	<6	1368.62	normal						
95	33	M	6556/11	1.1	30	21	8	4.1	3.9		negative	undetected	545.85	CLD				GIVx		
96	56	M	1674/11	1.5	30	21	7	4.7	3.3		negative	412	2265.3	CLD				GIIVx		
97	70	F	6213/12	1.6	42	40	6.2	4.1	2.1		negative	67	1834.9	fatty liver						
98	37	M	3048/14	1.3	36	42	6.9	3.9	3		negative	2240	4121.11	normal						
99	24	M	2975/14	6.9	165	43	7.9	4.2	3.7		negative	18.6	8162.09	normal						
100	33	M	2550/14	1.9	23	22	7.3	4.3	3		negative	43	879.9	normal						
101	49	F	2890/14	5	204	588	6.8	3.6	3.2		negative	94.7	196.33	normal						
102	49	M	3219/11	0.6	40	41	6.9	4.4	2.5		negative	9.43	1851.94	normal						
103	52	F	2766/14	0.9	19	16	8	4.2	3.8		negative	186	1650.18	normal						
104	18	M	4066/13	2	24	25	7	4.2	2.8		negative	152	6284.92	normal						
105	42	F	3638/14	1	22	2.6	7.4	4.4	3		negative	<6	7749	fatty liver						
106	55	M	2957/14	1.4	36	34	7.6	3.8	2.9		negative	238	547.11	normal						
107	55	F	4010/13	1.1	36	48	6.9	4.2	2.7		negative	undetected	570.39	normal						
108	22	F	2947/14	1.1	25	19	7.8	4.7	3.1		negative	<6	4886.76	normal						
109	35	F	3865/14	0.89	39	40	6.5	3.5	3		negative	19.3	987.07	fatty liver						
110	18	F	3608/14	1.3	30	25	7.3	4.1	3.2		negative	undetected	281055.77	normal						
111	23	F	3533/14	0.7	32	42	5.5	3.2	2.3		negative	<6	21838.05	normal						
112	44	M		1	41	38	6	3.8	2.2		negative	undetected	572.11	normal						
113	56	M		0.87	30	23	6.7	3.6	3.1		negative	120000	3913.07	fatty liver						
114	26	F		0.92	29	39	7.2	4.2	3		negative	<6	17281.13	fatty liver						
115	66	M		1.2	35	40	6.9	3.9	3		**reactive**	>110000000	32963.68	normal						
116	25	M		1	29	39	7.8	4.9	2.9		negative	1620	7661.1	normal						

117	33	M		0.86	30	41	6.9	4	2.9		negative	undetected	0.32	normal						
118	31	F		1.6	39	31	6.2	4	2.2		negative	116	2240.36	normal						
119	40	F		1.3	36	29	6.7	3.9	2.8		negative	1990	1255.83	normal						
120	49	M		0.92	40	36	7.6	4.6	3		negative	1720	442.05	normal						
121	42	F		1.1	32	40	7.1	4.2	2.9		negative	<6	817.2	normal						
122	40	M		1.4	29	33	6.7	3.5	3.2		negative	undetected	3934.71	normal						
123	20	F		0.99	26	39	6.7	3.9	2.8		negative	2790	7815.05	normal						
124	56	F		0.79	45	41	6.2	3	3.2		negative	806	3780.89	fatty liver						
125	51	M		1.3	25	31	7	4.8	2.2		negative	<6	439.84	normal						
126	31	M		1.6	49	44	6.6	3.3	3.3		negative	2280	61850.8	fatty liver						
127	54	M	3385/11	0.7	77	71	5.5	2.5	3		negative	undetected	2033.57	CLD						
128	39	M	6784/09	7.3	216	263	5.9	2.3	3.6		negative	undetected	273.44	CLD				GIVx		
129	27	F		1	43	39	7.5	4.4	3.1		negative	189	16782.27	fatty liver						
130	31	M		1.4	37	27	7.9	4.2	3.7		negative	1120	3077.99	normal						
131	37	M		0.89	35	27	6.9	4	2.9		negative	2240	4121.11	normal						
132	70	M	5722/12	1.4	24	32	5.2	2.2	3		negative	<6	363.3	CLD						
133	37	M	2977	2.4	115	86	6	3.2	3.4		**reactive**	131000	65300	CLD				GIIVx		
134	40	F	3755/14	0.5	33	44	7.2	4.5	2.7		negative	1555826	23400	normal						
135	45	M	4294/14	0.78	26	31	7.4	4.4	3		negative	43.5	789.61	fatty liver						
136	37	F	4480/14	1.1	32	28	8	4.8	3.2		negative	<6	379.6	normal						
137	48	F	4474/14	1.6	37	29	6.7	3.7	3		negative	32.3	769.54	normal						
138	21	F	4059/14	0.7	33	27	7.4	4.1	3.3		negative	168	16166.8	normal						
139	24	M	7627/13	1	65	88	8.9	4.9	4		**reactive**	1844.27	110000	fatty liver						
140	28	M	4228/14	0.7	43	69	7.3	4.4	2.9		negative	2930	9589.65	normal						
141	18	F	3608	1.3	30	25	7.3	4.1	3.2		negative	undetected	281055.77	normal						
142	50	M	7753/13	2.5	830	929	8.7	3	5		**reactive**	109000000, 110000000	9000, 13000	fatty liver						
143	25	F	2606/14	0.7	30	23	6.7	4.5	2.2		**reactive**	110000000	20461	normal						
144	24	F	4366/14	1	26	20	7.1	3.9	3.2		negative	6.13	2114.32	normal						
145	23	F	7136/13	1	35	30	7	3.9	3.1		negative	9.89	7594.96	normal						
146	22	M	4362/14	0.79	35	27	6.4	4.1	2.3		negative	246	6031.68	normal						
147	23	M	4268/14	3.4	465	541	7.2	4.2	3		**reactive**	>110000000	>100000	normal						
148	25	F		0.7	21	27	6	3.5	2.5		negative	<6	10510.83	normal						
149	24	F		1.4	30	36	7.3	4.3	3		negative	1070	3443	normal						
150	32	F	4323/14	1.3	28	19	6.1	3.2	2		negative	13100000	7805.17	normal						
151	47	M		0.89	18	27	6.7	4	2.7		negative	761	1120.9	normal						
152	46	M		1.4	37	26	8.1	4.9	3.2		**reactive**	5270000	4104.15	fatty liver						
153	43	F	4613/14	0.7	29	27	6.6	4.6	2		negative	648	9705.25	fatty liver						
154	31	M	4596/14	1.5	53	28	6.9	4.9	2		negative	16.4	4931.99	fatty liver						
155	18	M	4879/14	9.6	1280	2430	7.2	4.3	2.9		**reactive**	>110000000	100000	normal						
156	30	F	4995/14	0.98	22	40	6.8	4	2.8		negative	45	1093.8	fatty liver						
157	38	M	3011/13	1	19	31	5.4	3.4	2		negative	<6	68.8	normal						
158	40	M	6901/11	2.1	54	30	6.3	3.9	2.4		negative	undetected	572.81	normal						
159	55	F	4999/14	1.3	32	56	7.8	4.5	3.3		negative	415.3	1865.4	fatty liver						
160	40	M	4933/14	0.54	29	29.2	6	3.7	2.3		negative	736	1009.42	normal						

S.No	Name	Age/Sex	Diagnosis	CTP Score	HBeAg	nl				HBV DNA IU/ml			treatment		
						baseline	12wks	24wks	36wks	baseline	12wks	24wks	36wks		
1	lokeshwari	22	Active CHB	NA	**positive**	71116.89	66820	63078.75	59876	>1,10000000	52061.6	80,600	72389	tenofovir	
2	satish	18	active CHB	NA	**positive**	15341.82	18317.08	11713.2	10823	48,000,000	UD	UD		tenofovir	
3	gopal	3	active CHB	NA	**positive**	>1,00,000	80,456.26	74658.8	71166	>110,000,000	63074.61	92624	80509	lamivudine	
4	krbakaran	18	active CHB	NA	**positive**	27,400	16240	11251	96827	>110,000,000	92426	71426	82368	tenofovir	
5	thulasimma	12	active CHB	NA	**positive**	4433.84	4086.64	2893		42700	21800	30650		tenofovir	
6	kalliammal	37	active CHB	NA	**positive**	6792	5640	3890	2658	3610	1800	876	658	tenofovir	
7	ıravan muth	44	CLD	CTP A	negative	4231.23	2650	1685	86	5540	3580	2800	<6	telbuvidine	
8	murugesh	27	CLD	CTP A	negative	6423.12	2982.82	1089.22	146	4640	223	62.34	UD	telbuvidine	
9	sandhya	6	active CHB	NA	**positive**	>100,000	73492	59876	42768	110000	25700	16800	4700	lamivudine	
10	suman	15	active CHB	NA	**positive**	>100000	100000	89600		>1100000	131000	96700		telbuvidine	
11	moses	47	DCLD	CTP B	negative	367.37	258.9	189.8	109.3	95.5	<6	UD	UD	telbuvidine	
12	hokkanatha	27	active CHB	NA	negative	14977.6	10846	6839.7	3362.9	11189	6570	3200	867	telbuvidine	
13	anbalagan	50	acute flare	NA	negative	9000	13650	8650	6670	>10000000	>110000000	189000	96500	telbuvidine	
14	raje	65	active CHB	NA	negative	2062.28	987.67	589	268	81300	56400	19800	8870	telbuvidine	
15	sarasu	50	CLD	CTP A	negative	14881.6	7712.16	2874.2	759.16	>110000000	1060000	76500	29800	telbuvidine	
16	kothandan	51	CLD	CTP A	negative	4238.57	2033.57	1720.05	957.5	2539033	165000	54200	UD	entacavir	
17	sekar	47	active CHB	NA	negative	3989.19	1876.19	1099.9	658	701000	90500	8760	250	telbuvidine	
18	engalvaray	61	CLD	CTP A	negative	36500	16230	1385	886	15100000	198000	1040	56.8	tenofovir	
19	mani	34	active CHB	NA	negative	33961.75	28957.75	19860	11798	381000	297000	109000	87600	tenofovir	
20	evasundara	37	active CHB	NA	negative	3568.12	3090	2668.2	1969	131000	99700	50750	33500	tenofovir	
21	manju	25	active CHB	NA	**positive**	20461	9892.6	4682	2279	110000000	108900	60460	11590	tenofovir	
22	murugan	43	CLD	CTP A	negative	1211.78	782.22		178	5030	3800		1070	telbuvidine	
23	munwar	24	active CHB	NA	**positive**	20165	9957.28	5749	1020	110000000	99700	10650	2370	telbuvidine	
24	annalakshm	27	active CHB	NA	negative	11255.5	8690.5	5909	2305.5	184,000	65200	11800	3350	tenofovir	
25	ramesh	27	active CHB	NA	negative	4632.25	4079.2	3276	1976.25	7889	5980	3688	1690	tenofovir	
26	venkatesh	40	active CHB	NA	negative	96500	52486	18600	10040	1,555,826	196500	100000	67800	tenofovir	